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Dear Joshua:

I am enclosing a draft of a communication for Evelyn Witkin's MGB. It gives a pretty fair picture of my results with complementaries. To be more specific, the failures might be due to incompatible genes in the new pair of strains, because even in simple crosses on MTL they did not yield $P^+B_1^+$ recombinants as had the original strains; the background growth on MTL was heavy in all cases. However, back-crosses of each new parent with the opposite original parent failed to yield any complementaries. All or nearly all failures, including the back-crosses, were distinguished by an early growth of background (parentals) in the complementary MTL plates, and these colonies showed little or no change in their requirements when tested. Washed agar did not help. It is also difficult to understand why inhibitor selection was so fruitless, especially when I selected for $Az^rB_1^+$. In that particular experiment the $Az^rB_1^-$ parent grew out slowly in the complementary plates and there was no other growth. If incompatible genes were responsible for the earlier failures, the same genes could not account for this failure because one locus was completely different.

The colonies from which I screened the complementaries came, mainly, from pretty populous plates: two had about 70 colonies each, one had about 140 and another 230. I can't correlate the yield with the density of the inocula because I neglected to record the data that way. The many unsuccessful experiments nearly all involved plates with smaller counts, mostly less than 50. Yet I am sure that I obtained some complementaries from plates of 70 colonies or less in the successful experiments, and failed to get any from plates with over 200 colonies in one of the unsuccessful experiments.

You will understand from this why I hesitate to make a splash with the complementaries. Bernie thinks I ought to continue the work in my December vacation: try to clear up some of the doubt and write a paper for official publication. I am pessimistic about the value of such a month, and I was planning to do some work in medical genetics at that time. But I guess he is correct, and I also feel obligated to follow his advice and write up my results ~~as~~ to-date as though for publication. This letter is a sort of preview for such a paper.

With reference to the complementary selections and mapping, you must be aware that nutritionally complementary selections need not imply complementarity for segregation of markers. I was selecting for $M^+T^+L^+$ vs $P^+B_1^-$, not for $M^+T^+L^+$ vs $M^-T^-L^-$. But among my complementaries there were a few $M^-T^-L^-P^+B_1^+$, and they did resemble the $M^+T^+L^+P^+B_1^-$ with respect to most of the fermentations and Streptomycin resistance. There are not enough of them, however, to refute the linear hypothesis for those factors. The table at the end of the letter describes 5 of the complementaries.

The control series of $P^+B_1^+$ that I obtained in two experiments has such a high proportion of T^+ that no linear scheme fits the data unless I assume some selection for T^+ . However, the $P^+B_1^+$ complementaries screened from $M^+T^+L^+$ colonies, only 26 in number, fit the map very nicely. This is illustrated by the following:

Observed cross-over percentages

segment involved	$B_1 - M$	$M - P$	$P - LT$
Recombinant series: $M^+T^+L^+$	8	38	62
$P^+B_1^+$ (complementaries)	35	65	27

The two outside segments both give approximately the same correction for converting the middle segment to absolute distance: $27/62 \times 38 = 16.5$ $8/35 \times 65 = 15$. Unfortunately, the complementaries are not a random group of $P^+B_1^+$, but are necessarily non-prototrophs. Complete prototrophs should not be very numerous in ~~such~~ a random series, however.

Despite the partial selection for T^+ , the $P^+B_1^+$ control set agrees with the $M^+T^+L^+$ data on proportions of the component distances within the selected segments.

I'm sorry I didn't make myself clear about the selections on $P B_1$ medium supplemented with small amounts of MTL. The supplement was 20 or 30 mγ of each per cc. To illustrate, I made the two back-crosses simultaneously, ~~and~~ spreading each on several $P B_1$ plates and on several $P B_1 mtl$. The supplement made only a microscopic difference in the background growth, but the prototroph colonies grew sooner and larger on the enriched plates, averaging 43.5 ± 11 each as against 26.6 ± 4.5 on the unsupplemented plates. The difference is only about 1.5 times its s.d., but it assumes significance in conjunction with the fact that about $\frac{1}{2}$ of the excess of recombinants in the supplemented plates were P^- . The discrepancy in M-P crossover percentages for the two kinds of plate has a X^2_1 of 5.17. I think this warrants

the conclusion that in the presence of traces of the growth factors, some $M^+T^+L^+$ recombinants survive that would not have been detected on unsupplemented medium. In this experiment, these were largely prolineless recombinants.

This is a striking illustration of the complexity of prototroph selection. Other examples in my experience are the high frequency of T^+ among $P^+B_1^+$ recombinants selected on MTL, and the failure to get any $P^+B_1^+$ recombinants on MTL from the heavily-marked strains. It suggests that linkage data should be based on prototrophs obtained by a method that gives a maximum yield of recombinants. It also suggests the presence of several significant factor-differences in the two strains besides those recognized. Stocks used should perhaps be freshly obtained from a common parent, or should be made isogenic by repeated crosses using 2 different combinations of selectors alternately in successive generations.

A set of 100 $P^+T^+L^+$ recombinants, which I described to you June 1, confirms the relative lengths of B_1M and MP , but gives the total segment a much lower absolute value: $5+7$ instead of $8+15$ ($X^2_2 = 7.6$). Again in this series, within the selected segment we find confirmation of the linear order and ~~and~~ general proportions indicated by the $M^+T^+L^+$ data, with the exception of lactose. You will see from the diagrams below that both enriched and unenriched $M^+T^+L^+$ series were ambiguous with respect to lactose, (although Ia and V_6 , on left and right of P , respectively, placed lactose definitely to the left of P in the enriched series). I believe your data place lac to the right of V_6 (in W-677). Certainly the behavior of lactose is suspicious; I still think all the fermentations may be affected by something other than the unit factors.

Linkage maps from 3 series of recombinants.

$M^+T^+L^+$ (about 150 selected on PB_1 on PB_1mtl)	38	P	51	
	35	Lac	47	V_1
$M^+T^+L^+$ (about 250 selected on PB_1)	23.5	P	49	
	23	Lac	48	V_1
$P^+T^+L^+$ (100 selected on MB_1)	7	P	77	
	14	Lac	70	V_1

The other anomalies in the $P T L$ series that I mentioned in my earlier letter suggested that xylose, Ia^r , ara , etc sometimes show repulsion from LT without being linked to loci near P . I now find that practically all triple cross-overs in my experiments are like this; i.e. two of the three cross-

overs represent the misbehavior of a single marker. But I think my methods are sufficiently inaccurate to account for all such anomalies on the basis of errors in testing. Examples of these doubtful triples are seen in #1 and #5 in the last table.

In that connection I once suggested analyzing triple cross-overs in comparison with single cross-overs for locating deleterious mutations and for estimating the absolute linkage distances. Since ~~all but one~~ of my triples belong to the (nearly) above doubtful category, the method is impractical. But incidentally, interference does not seem to be very important, because among 115 $M^+T^+L^+$ recombinants with cross-overs between M and P, 5% also crossed over between M and B_1 , close to the expected 8%.

Detailed analysis of some M T L recombinants and the complementaries screened from them. (The five obtained from the cross involving the heavily marked strains) X70g)

Map distances obtained from prototrophs on PB_1mtl medium, corrected to absolute values. (all triple cross-overs used for mapping)

length of segments:	8	7	13	3	4	20	5	4	
markers:	B_1	M	I	Lac	P	V_6	V_1	Az	IT
Parental D100	+	-	-	+	-	+	-	-	++
W-1234Ia ^r	-	+	+	-	+	-	+	+	--
principle #1	-	+	+	+	-	-	-	-	++
complementary #1	+	-	-	-	+	-	+	+	--
#2	-	+	-	+	-	+	-	-	++
#3	+	-	+	-	+	-	+	+	++
#4	-	+	+	-	+	-	+	+	++
#5	+	-	+	+	+	-	-	-	++
	+	-	-	-	+	-	-	-	+-

*principles were
 $M^+T^+L^+P^+B_1^-$*

Excepting the ~~five~~³ lac⁺ principles and one man + complementary, these 10 strains were negative for all fermentations (not including mal, which I found hard to score), and they were all Streptomycin sensitive like W1234. Note the apparently random occurrence of cross-overs; one would expect a tendency toward reciprocal crossing-over if they were sister-segregants. These are representative, but of course the others have fewer markers.

I doubt if my data on complementary selections are extensive enough to publish in PNAS as you suggested, especially because

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the most important data come from the 26 "complementaries", and if I mentioned them I'd have to explain how ~~they~~ they were obtained. Of course, I'd like to get the whole thing off my chest, as soon as possible, but I might regret it later.

I think this, together with the abstract for MGB, gives you a pretty good summary of my year's work on E. coli, except for the negative findings on multiple loci for drug resistance. I think I have explained all my reservations, so there should be no danger in your drawing from it any conclusions that appear to be justified. I think that amounts to zero, except that K-12 genetics has many pitfalls ! I'll certainly not be hurt if you agree with me and refrain from mentioning any of this in September.

I have a real urge to explore the subject further, but I am more a crusader than a scientist, so I doubt if I could be content in bacterial genetics. And if the war spreads, I may even spend a good part of my life as an army or navy doctor.

I knew that you and Esther were going to California for the summer, so it was plain stupid of me to address 2 letters to Madison. I hope you are both having a very happy summer.

Sincerely yours,

Gordon